



Standard Test Method for Evaluation of Cytotoxicity of Nanoparticulate Materials in Porcine Kidney Cells and Human Hepatocarcinoma Cells¹

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1. Scope

1.1 This test method provides a methodology to assess the cytotoxicity of suspensions of nanoparticulate materials in porcine proximal tubule cells (LLC-PK1) and human hepatocarcinoma cells (Hep G2) which represents potential target organs following systemic administration

1.2 This test method is part of the *in vitro* preclinical characterization cascade.

1.3 This test method consists of a protocol utilizing two methods for estimation of cytotoxicity, 3-(4,5-Dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) reduction and lactate dehydrogenase (LDH) release.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 ASTM Standards:²

F813 Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F1877 Practice for Characterization of Particles

F1903 Practice for Testing For Biological Responses to Particles *In Vitro*

¹ This test method is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.03 on Environment, Health, and Safety.

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² For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

2.2 ISO Standard:³

ISO 10993-5 Biological Evaluation of Medical Devices: Part 5 Tests for *in vitro* Cytotoxicity

3. Terminology

3.1 Abbreviations:

3.1.1 **APAP**—acetaminophen- positive control

3.1.2 **DMSO**—dimethyl sulfoxide

3.1.3 **DMEM**—Dulbecco's modified eagles media

3.1.4 **Hep G2**—human hepatocarcinoma cells

3.1.5 **LDH**—lactic dehydrogenase

3.1.6 **LLC-PK1**—porcine proximal tubule cells

3.1.7 **LPS**—lipopolysacchride, bacterial endotoxin

3.1.8 **MTT**—3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide

3.1.9 **Physiologic solution**—isotonic with a pH 7.2 ± 0.2

4. Summary of Test Method

4.1 Nanoparticulate test materials in suspension in cell culture media and appropriate controls are added to cell cultures. The release of LDH indicates membrane damage and the diminution of MTT reduction indicates loss of cell viability. These are quantitative indicators of cytotoxicity. Aseptic procedures are required.

5. Significance and Use

5.1 Assessing the propensity of a nanomaterial to cause cytotoxicity to the cells of a target organ can assist in preclinical development.

5.2 The standard historical cytotoxicity testing of materials and extracts of materials has used fibroblasts and is well documented in Practice **F813**, Test Method **F895**, and ISO 10993-5. The use of macrophages and micron size particles has also provided information on cytotoxicity and stimulation using Practice **F1903**.

³ Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, <http://www.ansi.org>.